

CLAIMS

1. A method of distinguishing between rice varieties, comprising the following steps (a) and (b):

5 (a) determining the type of a nucleotide at a position according to any of the following (1) to (28) in the rice genome, or a nucleotide on the complementary strand that composes a base pair with the nucleotide at the position:

- (1) position 593 in the nucleotide sequence of SEQ ID NO: 1,
- 10 (2) position 304 in the nucleotide sequence of SEQ ID NO: 2,
- (3) position 450 in the nucleotide sequence of SEQ ID NO: 3,
- (4) position 377 in the nucleotide sequence of SEQ ID NO: 4,
- (5) position 163 in the nucleotide sequence of SEQ ID NO: 5,
- (6) position 624 in the nucleotide sequence of SEQ ID NO: 6,
- 15 (7) position 534 in the nucleotide sequence of SEQ ID NO: 7,
- (8) position 358 in the nucleotide sequence of SEQ ID NO: 8,
- (9) position 475 in the nucleotide sequence of SEQ ID NO: 9,
- (10) position 323 in the nucleotide sequence of SEQ ID NO: 10,
- (11) position 612 in the nucleotide sequence of SEQ ID NO: 11,
- 20 (12) position 765 in the nucleotide sequence of SEQ ID NO: 12,
- (13) position 571 in the nucleotide sequence of SEQ ID NO: 13,
- (14) position 660 in the nucleotide sequence of SEQ ID NO: 14,
- (15) position 223 in the nucleotide sequence of SEQ ID NO: 15,
- (16) position 247 in the nucleotide sequence of SEQ ID NO: 16,
- 25 (17) position 163 in the nucleotide sequence of SEQ ID NO: 17,
- (18) position 421 in the nucleotide sequence of SEQ ID NO: 18,
- (19) position 178 in the nucleotide sequence of SEQ ID NO: 19,
- (20) position 141 in the nucleotide sequence of SEQ ID NO: 20,
- (21) position 480 in the nucleotide sequence of SEQ ID NO: 21,
- 30 (22) position 481 in the nucleotide sequence of SEQ ID NO: 22,
- (23) position 131 in the nucleotide sequence of SEQ ID NO: 23,
- (24) position 510 in the nucleotide sequence of SEQ ID NO: 24,
- (25) position 248 in the nucleotide sequence of SEQ ID NO: 25,
- (26) position 92 in the nucleotide sequence of SEQ ID NO: 26,
- 35 (27) position 743 in the nucleotide sequence of SEQ ID NO: 27,

and

- (28) position 552 in the nucleotide sequence of SEQ ID NO: 28,
and
(b) relating the type of the nucleotide determined in step (a) to
a variety of rice.

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2. The method of claim 1, which distinguishes the type of a nucleotide
by using a polymorphic marker characterized by a mutation of any
of the following (1) to (28) in the rice genome:

- (1) the nucleotide at position 593 in the nucleotide sequence
10 of SEQ ID NO: 1 is T,
(2) the nucleotide at position 304 in the nucleotide sequence
of SEQ ID NO: 2 is T,
(3) the nucleotide at position 450 in the nucleotide sequence
of SEQ ID NO: 3 is A,
15 (4) the nucleotide at position 377 in the nucleotide sequence
of SEQ ID NO: 4 is C,
(5) the nucleotide at position 163 in the nucleotide sequence
of SEQ ID NO: 5 is C,
(6) the nucleotide at position 624 in the nucleotide sequence
20 of SEQ ID NO: 6 is C,
(7) the nucleotide at position 534 in the nucleotide sequence
of SEQ ID NO: 7 is C,
(8) the nucleotide at position 358 in the nucleotide sequence
of SEQ ID NO: 8 is G,
25 (9) the nucleotide at position 475 in the nucleotide sequence
of SEQ ID NO: 9 is G,
(10) the nucleotide at position 323 in the nucleotide sequence
of SEQ ID NO: 10 is A,
(11) the nucleotide at position 612 in the nucleotide sequence
30 of SEQ ID NO: 11 is A,
(12) the nucleotide at position 765 in the nucleotide sequence
of SEQ ID NO: 12 is T,
(13) the nucleotide at position 571 in the nucleotide sequence
of SEQ ID NO: 13 is T,
35 (14) the nucleotide at position 660 in the nucleotide sequence
of SEQ ID NO: 14 is G,

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- (15) the nucleotide at position 223 in the nucleotide sequence
of SEQ ID NO: 15 is A,
- (16) the nucleotide at position 247 in the nucleotide sequence
of SEQ ID NO: 16 is A,
- 5 (17) the nucleotide at position 163 in the nucleotide sequence
of SEQ ID NO: 17 is A,
- (18) the nucleotide at position 421 in the nucleotide sequence
of SEQ ID NO: 18 is C,
- (19) the nucleotide at position 178 in the nucleotide sequence
10 of SEQ ID NO: 19 is G,
- (20) the nucleotide at position 141 in the nucleotide sequence
of SEQ ID NO: 20 is G,
- (21) the nucleotide at position 480 in the nucleotide sequence
of SEQ ID NO: 21 is C,
- 15 (22) the nucleotide at position 481 in the nucleotide sequence
of SEQ ID NO: 22 is C,
- (23) the nucleotide at position 131 in the nucleotide sequence
of SEQ ID NO: 23 is C,
- (24) the nucleotide at position 510 in the nucleotide sequence
20 of SEQ ID NO: 24 is A,
- (25) the nucleotide at position 248 in the nucleotide sequence
of SEQ ID NO: 25 is T,
- (26) the nucleotide at position 92 in the nucleotide sequence
of SEQ ID NO: 26 is C,
- 25 (27) the nucleotide at position 743 in the nucleotide sequence
of SEQ ID NO: 27 is G, and
- (28) the nucleotide at position 552 in the nucleotide sequence
of SEQ ID NO: 28 is T.
- 30 3. The method of claim 1 or 2, comprising the following steps (a) to
(c):
- (a) preparing DNA from a test rice,
- (b) amplifying a DNA comprising a nucleotide in a position
of any of (1) to (28) of claim 1, or a nucleotide in the
35 complementary strand composing a base pair with the nucleotide
at the position, and

(c) determining the nucleotide sequence of the amplified DNA.

4. The method of claim 1 or 2, comprising the following steps (a) to (d):

- 5 (a) preparing DNA from a test rice,
(b) digesting the prepared DNA with a restriction enzyme,
(c) fractionating the DNA fragments by size, and
(d) comparing the size of the detected DNA fragment with a control.

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5. The method of claim 1 or 2, comprising the following steps (a) to (e):

- (a) preparing DNA from a test rice,
(b) amplifying a DNA comprising a nucleotide in a position
15 of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,
(c) digesting the amplified DNA with a restriction enzyme,
(d) fractionating the DNA fragments by size, and
20 (e) comparing the size of the detected DNA fragment with a control.

6. The method of claim 1 or 2, comprising the following steps (a) to (e):

- 25 (a) preparing DNA from a test rice,
(b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,
30 (c) denaturing the amplified DNA into single-stranded DNAs,
(d) fractionating the denatured single-stranded DNA on a non-denaturing gel, and
(e) comparing the mobility of the fractionated single-stranded DNA on the gel with a control.

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7. The method of claim 1 or 2, comprising the following steps (a) to

(f):

(a) preparing DNA from a test rice,

(b) synthesizing two different oligonucleotide probes labeled with a reporter fluorescence dye and quencher fluorescence dye, where an oligonucleotide is complementary to a proximal nucleotide sequence comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(c) hybridizing the DNA prepared in step (a) with the probe synthesized in step (b),

(d) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(e) detecting the emission of reporter fluorescence, and

(f) comparing the emission of reporter fluorescence detected in step (e) with a control.

8. The method of claim 1 or 2, comprising the following steps (a) to (h):

(a) preparing DNA from a test rice,

(b) synthesizing a probe in which a sequence complementary to the 3'-flanking nucleotide sequence comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position, is combined with a totally unrelated sequence,

(c) synthesizing a probe that is complementary to the 5'-flanking region comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(d) hybridizing the probe synthesized in step (c) with the DNA prepared in step (a),

(e) digesting the hybridized DNA in step (d) with a

single-stranded DNA cleaving enzyme, and dissociating a part of the probe synthesized in step (b),

(f) hybridizing the dissociated probe in step (e) with a probe for detection,

5 (g) enzymatically digesting the hybridized DNA in step (f), and measuring the fluorescence intensity thus generated, and

(h) comparing the fluorescence intensity measured in step (g) with a control.

10 9. The method of claim 1 or 2, comprising the following steps (a) to (f):

(a) preparing DNA from a test rice,

15 (b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(c) denaturing the amplified DNA into single-stranded DNAs,

(d) separating only one strand from the denatured single-stranded DNAs,

20 (e) performing an elongation reaction from near a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position, whereby the reaction elongates one nucleotide at a time, then enzymatically illuminating the generated pyrophosphate, and measuring the intensity of the illumination, and
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(f) comparing the fluorescence intensity measured in step (e) with a control.

30 10. The method of claim 1 or 2, comprising the following steps (a) to (f):

(a) preparing DNA from a test rice,

35 (b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(c) synthesizing a probe complementary to a nucleotide sequence comprising a sequence covering up to a nucleotide adjacent to a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(d) performing a single nucleotide extension reaction in the presence of fluorescently labeled nucleotides, using the DNA amplified in step (b) as a template, and the primer synthesized in step (c),

(e) measuring the fluorescence polarization, and

(f) comparing the fluorescence polarization measured in step (e) with a control.

11. The method of claim 1 or 2, comprising the following steps (a) to (f):

(a) preparing DNA from a test rice,

(b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(c) synthesizing a primer complementary to a nucleotide sequence comprising a sequence covering up to the nucleotide adjacent to a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,

(d) performing a single nucleotide extension reaction in the presence of fluorescently labeled nucleotides, using the DNA amplified in step (b) as a template, and the primer synthesized in step (c),

(e) determining the nucleotide variety used in the reaction of step (d) using a sequencer, and

(f) comparing the nucleotide determined in step (e) with a control.

12. The method of claim 1 or 2, comprising the following steps (a) to (d):

- (a) preparing DNA from a test rice,
- (b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,
- (c) measuring the molecular weight of the DNA amplified in step (b) using a mass spectrometer, and
- (d) comparing the molecular weight measured in step (c) with a control.

13. The method of claim 1 or 2, comprising the following steps (a) to (f):

- (a) preparing DNA from a test rice,
- (b) amplifying a DNA comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position,
- (c) providing a substratum on which a nucleotide probe is immobilized,
- (d) contacting the DNA of step (b) with the substratum of step (c),
- (e) detecting the strength of hybridization between the DNA and the nucleotide probe immobilized on the substratum, and
- (f) comparing the strength detected in step (e) with a control.

14. The method of any of claims 1 to 13, further comprising the following steps (a) and (b):

- (a) disrupting a rice seed in an alkaline aqueous solvent, and
- (b) extracting rice genomic DNA from the seed disrupted in step (a).

15. The method of claim 14, wherein the rice seed is polished.

16. A primer for distinguishing between rice varieties, wherein the

primer is (a) an oligonucleotide for amplification of a DNA region comprising a nucleotide in a position of any of (1) to (28) of claim 1 in the rice genome, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position, or (b)
5 an oligonucleotide comprising a nucleotide sequence complementary to a sequence covering up to a nucleotide adjacent to a position of any of (1) to (28) of claim 1 in the rice genome, or a nucleotide in the complementary strand composing a base pair with the nucleotide at the position.

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17. An oligonucleotide for distinguishing between rice varieties, wherein the oligonucleotide hybridizes with a DNA region comprising a nucleotide in a position of any of (1) to (28) of claim 1, or a nucleotide in the complementary strand composing a base
15 pair with the nucleotide at the position, comprising at least 15 nucleotides.

18. A kit for distinguishing between rice varieties, comprising the oligonucleotide of claim 16 or 17.

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19. The kit of claim 18, further comprising an alkaline aqueous solvent.